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The effects of genetic selection for survivability and productivity on chicken physiological homeostasis

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Genetic selection is an important breeding tool that can be used for improving the animal's coping capability to modern production environments or for increasing economic benefits. However, over the past five decades, commercial breeding programmes have primarily concentrated on traits directly related to productivity. As a result those breeding programmes ignore traits that may impact animal welfare. To address this issue, a selection programme termed "group selection" was developed. This method takes into account competitive interactions by emphasizing performance of the group, rather than the individual. Results from the current studies have showed that chickens' productivity and well-being can be improved at the same time. We further demonstrated an association between the selected line's survivability and productivity and its respective physiological characteristics. These findings indicate that group selection altered the chickens' physiological homeostasis which is reflected in the line's unique coping ability with intensified domestic environments. These changes in physiological homeostasis provide an opportunity to gain new insights for the development of interventions aimed at ameliorating the adverse impacts of the intensified poultry industry.

Keywords: group selection; chicken; social interactions; physiology; immunology

Abbreviations: CORT = Corticosterone; CNS = Central nervous system; DA = Dopamine; DβH = Dopamine-β-hydroxylase; EP = epinephrine; DXL = Commercial Dekalb XL line; HA line = chicken line selected for high antibody response to SRBC; HPA axis = Hypothalamic-pituitary-adrenal axis; H:L = Heterophil:lymphocyte ratio; KGB = Kinder Gentler Bird selected for high productivity and longevity; LA line = chicken line selected for high antibody response to SRBC; LH = luteinizing hormone; LHRH = luteinizing hormone-releasing hormone; MBB = Mean Bad Bird selected for low productivity and longevity; MHC = Major histocompatibility complex; NE = norepinephrine; SRBC = Sheep red blood cell; TH = tyrosine hydroxylase; 5-HT = Serotonin; 5-HIAA = 5-hydroxyindoleasetic acid

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Introduction

Both nature (genetics) and nurture (environment) influences an animal's coping strategies to stressors, which in turn are reflected in its productivity and survivability. Genetic selection is an important tool for improving an animal's coping capability to the changes of its internal and external environmental factors. These changes are often intensified with modern farm animal practices. However, over the past five decades, in most breeding programmes, only traits directly related to productivity were considered. As a result, traits that were selected may have had a negative impacted on welfare.

In commercial poultry operations for egg production, birds are kept in cages ranging in size from 5 to 9 birds or up. In those environments, there are several consequences of ignoring animal welfare: 1) if productivity is correlated with competitive capability, then, the effect of selection for production is to increase competition; 2) increased competition has the effect of lowering productivity of other animals such as subordinates that are in direct contention, thus resulting in reduced (or negative) gains for productivity (as a group); and 3), genotype-genotype interactions (competition) invalidates the traditional Best Linear Unbiased Prediction animal model and negates many advantages of this technology and could in fact make it a liability as demonstrated by Muir (2005). For instance, through more than 20 years selection, egg production has increased significantly in the commercial Dekalb XL birds, while mortality due to aggression and cannibalism in non-beak-trimmed birds has also increased about 10-fold (Muir, 1996).

Recently, a selection programme termed "group selection" was introduced (Craig and Muir, 1996a, b; Muir, 1997, 2001, 2003; Cheng *et al.*, 2001 a, b; Muir and Schinckel, 2002, Muir, 2005). The innovation of the programme is that it allows selection on production traits but takes into account competitive interactions in a group setting. The programme turns "survival of the fittest" emphasized on the individuals to "survival of the adequate" in the group. Results from group selection studies with poultry have showed that productivity can be increased while at the same time well-being improved. This review represents a summary of current physiological studies of chickens developed in the selection programme and inference for development of strategies for improvement of animal well-being.

Development of the genetic lines

A randomly bred population of White Leghorns obtained from the North Central Regional Poultry Breeding laboratory (West Lafayette, IN 47906) was used in the selection programme. The line was originated crossing six commercial stocks in 1972 and randomly mated without conspicuous selection (Garwood *et al.*, 1980). The commercial DeKalb XL (DXL) line obtained from Dekalb Poultry Research (Dekalb, IL 60115) was one of the resources used to establish the original line. In the current study, as a control line, this original line was maintained as an unselected randomly mating without conscious selection and housed in single bird cages. The selected kinder gentler line (Kinder Gentler Bird, KGB) was established from the control line starting in 1982 on the basis of "family' or "group" performance for survival and egg production (Muir, 1996). During the selection each sire family was housed as a group in a multiple-bird cage and selected or rejected as a group based on production performance and longevity. Birds were not beak-trimmed and lights were at high intensity so as to allow expression of genetic variation for aggression, feather pecking, and cannibalism.

By the 7th generation selection progressed such that in multiple-bird cages, in comparison to the control line and DXL line, annual percent mortality of the KGB line was significantly reduced at 58 wk of age, *i.e.*, 20%:54%:89% (KGB:C:DXL) (*Figure 1a*) while production was significantly increased, *i.e.*, 60%:57%:49% (KGB:C:DXL) (*Figure 1b*). The higher mortality of the DXL line as compared to the unselected control line demonstrates that continued selection on productivity in single bird cages was detrimental to survival and well-being. The high survival of KGB line in relation to the unselected control line demonstrates that productivity and well-being can be simultaneously improved. These results demonstrate a classic genotype-environment interaction. Such interactions were predicted by the theories of Griffing (1967) and arguments of William (1966) whereby group adaptations require group selection and individual adaptations will be opposed to those of the group.

The 8th generation was produced from mating 1,248 hens with 312 roosters selected at random from 20-wk-old pullets and roosters of the 7th generation (Muir, 1996). Briefly, at 18 wk of age, 9,216 pullets were housed by half-sib family in 768 12-hen cages, which was the base population in the selecting of the 8th generation. After 52 wk of production (72 wk of age), birds from 12 cages with the highest group productivity (egg number) and the lowest mortality from cannibalism and flightiness, along with their full- and half-sib brothers, were selected for the next generation of KGB line. The mortality and production were further improved in the selected line (*Table 1*). To establish a comparison line, birds from 12 cages with the lowest group productivity and survivability, along with their full- and half-sib brothers, were used to establish a reverse selected MBB line (Mean Bad Bird) (*Table 1*).

Birds were randomly mated within each line, four hens per rooster, avoiding full or half-sib mating to reproduce the 9th generation of the KGB and the MBB line which were used as the genetic material for the current studies. Pullets of each genetic line were not beak-trimmed, and reared under the same conditions, hatched, vaccinated against Marek's and Newcastle disease, and maintained using standard management practices in raised wire cages up to 17 wk of age. To study genetic selection-induced physiological changes and to eliminate social stress effects, at 17 wk of age, birds from each line were randomly assigned to single-bird cages, each providing 1,085 cm^2 per bird. The birds still can contact each other, at least partially, through wired cages without social conflict, *i.e.*, fighting and cannibalism. Feed and water were provided for *ad libitum* consumption. Overhead lights were on daily from 0700 until 21:00 initially, and were increased by 15 min/wk until 16 h light daily.

Line differences in blood concentrations of catecholamines

Dopamine (DA), epinephrine (EP), and norepinephrine (NE) belong to the catecholamine family, which participate in a number of physiological and pathological processes, including regulation of domestic behaviour, production, and coping strategies to stressful conditions (Bell and Hepper, 1987; Haller *et al.*, 1997).

DOPAMINE

Under physiological conditions, DA is released centrally as a neurotransmitter, and is processed peripherally as a precursor of NE (Kuchel, 1991). Since DA cannot cross the blood-brain barrier, peripheral circulating DA could be either from adrenal cells or from leukocytes (Berquist *et al.*, 1998). However, during pathological conditions, DA can be released from adrenomedullary chromaffin cells, resulting in transformed adrenal cells from an adrenergic to a dopaminergic source (Snider and Kuchel, 1983). Abnormal blood and brain DA systems, such as increase of its concentrations and or its receptor density, have been associated with dysfunctional behaviour as well as with a decline in ability to cope with stress (Goldstein, 2003). In agreement with these findings, the KGB birds, had a significantly lower blood level of DA (*Table 2*, Cheng *et al.*, 2001a) and better and quicker adaptation to various stressors, such as social and heat stress (Hester *et al.*, 1996a, b). In addition, the lower blood DA concentrations in the KGB birds could be associated with their sedate and passive behaviours (Craig and Muir, 1996a, b). In contrast, the MBB birds had greater blood DA concentrations (*Table 2*), which could be linked to the specific reorganization of behaviours such as cannibalism, resulting in higher mortality (*Table 1*).

Higher blood concentrations of DA in the MBB birds combined with lower productivity (*Table 1 and 2*) are consistent with the hypothesis that the dopaminergic system is one of the main inhibitory neuronal systems that controls the development of the reproductive systems (Becu-Villalobos and Libertun, 1995) and productivity (Sotowska-Brochocka *et al.*, 1994). Each selected line's unique changes of DA concentrations indicate that selection for production and longevity has induced different effects on the physiological functions of the neuroendocrine systems, including the dopaminergic system. The changes of the dopaminergic system is a result of alternative functions in controlling productivity, such as endogenous DA inhibition of luteinizing hormone-releasing hormone (LHRH) (Contijoch *et al.*, 1992) and luteinizing hormone (LH) (Martin *et al.*, 1981).

Similar to the present findings, genotypic dependence in controlling animal domestic behaviour and reproduction has also been demonstrated in other species. Edens (1987) reported that, in Japanese quails, a higher concentration of DA was found in the brain of the birds which exhibited aggressive behaviour. Data from the current studies further suggest that DA plays a significant role in controlling aggressive behaviour and productivity. In the present study, strain selection may directly or indirectly influence the regulation of the dopaminergic system, and in turn, the activation of the DA system to favour survival behaviour in the KGB birds.

EPINEPHRINE AND NOREPINEPHRINE

Epinephrine is released almost exclusively from the adrenal medulla, with a small amount synthesized in the brain, while NE is released from sympathetic neurons, with a significant amount synthesized in the central nervous system (CNS), especially, in the hypothalamus (Bullock *et al.*, 1995). As "stress hormones", both EP and NE participate in regulation of emotion and motivation in response to stimulations. Changes in EP and NE levels, as well as the ratio of EP:NE have been used as indicators of well-being and capability to cope with stress (Goldstein, 1981; Dillon *et al.*, 1992). Consistent with this hypothesis, the present study showed that the changes in blood concentrations of EP and ratio of EP:NE in the KGB and MBB birds were observed along with differences in productivity and mortality due to cannibalism and flightiness (*Table 1 and 2*). The increased EP:EN ratio in the MBB birds was due to higher concentrations of EP, as there was no significant difference in the concentration of NE between the lines (*Table 2*). A similar up-regulation of EP concentration was found in the HL turkeys (Brown and Nestor, 1974) that were selected for great adrenal response to cold stress. Turkeys of the HL line, similar to the MBB birds, laid significantly fewer eggs, were hyperactive, and had poorer feed efficiency in comparison with the birds from its reverse selected LL line (lower adrenal response line). These data support the hypothesis that EP levels are reflective of an individual coping ability and associated with its behavioural patterns.

Cellular mechanisms for changing catecholamine concentrations, including increases in peripheral circulating DA, could be related to stress-induced imbalance of activities of enzymes involved in catecholamine metabolism (Kuchel, 1991). Stressors stimulate tyrosine hydroxylase (TH) activity and, at the same time, inhibit activities of the enzyme dopamine-β-hydroxylase (DβH) that converts DA to NE (Kuchel *et al.*, 1982). If DβH activation cannot keep pace with activation of TH, then DA synthesis predominates, and extra DA is released. Studied have shown that changes of TH activation can be induced by selection for domestic behaviour in animals (Dygalo *et al.*, 1988; Kulikov *et al.*, 1989), and that stress triggers differential regulation of gene expression for catecholamine biosynthetic enzymes found in the adrenal medulla (Nankova and Sabban, 1999).

Line differences in blood concentrations of serotonin

The role of serotonin (5-HT) is to modulate behavioural and physiological processes, including feeding, sexual, emotion, and social abilities (Pretorius, 2004; Shively and Bethea, 2004). Abnormalities of blood and brain 5-HT and its metabolite 5-hydroxyindoleasetic acid (5-HIAA), as well as the density of its receptors, have been used as major indicators to evaluate alterations in behavioural and physiological adaptability in response to intensified social environments, such as aggression and reproduction (Bell and Hobson 1994; Maswood *et al.*, 1998; Miczek *et al.*, 2002).

In the CNS, depletion or decrease of 5-HT concentrations and the ratio of 5-HIAA:5-HT have been implicated in dysfunctional behaviours, including aggressiveness and violence in human and animals (Higley *et al.*, 1996; Unis *et al.*, 1997; Parmigiani *et al.*, 1999), and cannibalism in rats (Barofsky *et al.*, 1983). In the peripheral system, however, the biological roles of 5-HT in behavioural adaptation are unclear. Conflicting changes in blood 5-HT concentrations have been found in association with behavioural dysfunctions, including aggressiveness (Hanna *et al.*, 1995; Moffitt *et al.*, 1998). The contradictory data from different investigations could be related to different genetic selection programmes, species, behavioural evaluations and stressors used, as well as duration and frequency of stressor presentation. The present data showed that higher blood 5-HT levels were associated with lower survivability in the MBB birds, which was possibly due to increased aggression (*Table 1 and 2*). Positive associations of blood 5-HT levels and aggressiveness were also found in adolescents with behavioural conduct disorder (Cook and Leventhal, 1996; Unis *et al.*, 1997), and in dominant male monkeys (Steklis *et al.*, 1986; Raleigh *et al.*, 1991). Values of blood 5-HT have also been used as a heritably stable biological parameter in rodents (Jernej and Cicin-Sain, 1990).

Whether the higher concentrations 5-HT in the blood of the MBB line would reflect similar changes in the brain is unclear, as 5-HT can not pass the brain-blood-barrier (Pietraszek *et al.*, 1992) and is regulated differently in the CNS and peripheral tissues (Lampugnani *et al.*, 1986; Pietraszek *et al.*, 1992). Previous studies have shown that supplemental tryptophan, a precursor of 5-HT, decreases aggression in feed-restricted male chickens (Shea *et al.*, 1991). Further studies are needed to determine whether different regulations of the peripheral and CNS systems are present in these lines.

The finding that the MBB birds, compared to the KGB birds, had higher concentrations of circulating 5-HT, but lower productivity (*Table 1 and 2*), is consistent with the reports that 5-HT has a tonic, inhibitory effect on sexual behaviour and reproduction (Sirotkin and Schaeffer, 1997), such as inhibition of LH secretion and ovulation in rodents (Nagatsuka, 1983; Morello *et al.*, 1992; Lorrain *et al.*, 1998). The effects of 5-HT on sexual behaviour were positively correlated to stimulation of the pre-optic area and median eminence of the hypothalamus where regulate reproductive activities (Gonzalez *et al.*, 1997). Although, at present, the cellular mechanisms that genetically regulate productivity between the selected lines are unclear, it may be the same as those found in rodents. There is evidence that the functions of the avian neuroendocrine system that controls stimulators are analogous to those in rodents (Harvey *et al.*, 1984). In addition, there are similar distributions of neurotransmitter receptors, including 5-HT receptors, in birds and mammals (Richfield *et al.*, 1987; Walker *et al.*, 1991).

Line differences in the adrenal system

Corticosterone (CORT) is released from the adrenal cortex, the rate of secretion being controlled by activation of the HPA axis via a double negative feedback loop. During pathologic conditions, the concentration of CORT can be increased by overriding the HPA control systems. Corticosterone, as one of the "stress hormones", has multifunctional roles in both normal and abnormal states, including regulation of the organism's behavioural patterns, coping styles and well-being (Savory and Mann, 1997; Haller *et al.*, 2000).

The concentrations of CORT were not found to be significant different, but there was a tendency for CORT concentration to be greater with heavier adrenal glands in KGB birds than in those of the MBB birds (*Table 3*, n=12, P=0.08 and P<0.05, respectively). The differences in the levels of CORT and adrenal weights between these lines could be the results of genetically different regulations of adrenal activities induced by selection. Similarly, Hester *et al.* (1996b) found no significant difference between the KGB and control lines on plasma CORT concentrations in response to acute heat stress. In their study, the lack of response may be due to acclimation caused by prior repeated stress, including social, cold, and fear stress. In a review of previous studies, Siegel (1995) suggested that concentrations of CORT may be a better indicator of acute or life-threatening stress.

The heavier adrenal glands and tendency for a greater CORT concentration in the KGB birds, as compared to the MBB, suggests that there may be an up-regulating the activity of the adrenal system in the KGB birds. There changes could be a part of defence mechanisms against environmental challenges, which may underlie their higher survivability, sedate and passive behaviours, and better coping to social, handling and environmental stressors (Hester *et al.*, 1996a, b; Muir and Craig, 1998). Similar to the findings, Siegel (1971) indicated that selection-induced adrenal hypertrophy may be an indicator of greater adaptation to stress.

Corticosterone response to stress in the KGB birds may also be related to its role as an immunomodulator. For example, infection Escherichia coli in chickens can be prevented by pre-treatment with CORT (Gross and Colmano, 1970). CORT can also protect the organism from damage by an excessive response of its immune system (Gross and Colmano, 1970; Munck *et al.*, 1984). Our data and others are consistent with the notion that one of the primary roles of endogenous glucocorticoids may be immunomodulation rather than immunosupression (Dhabhar *et al.*, 1994) and that moderate increases in levels of glucocorticoid may enhance immune functions (Stanulis *et al.*, 1997). Consistent with this hypothesis, the current study showed that the KGB birds had a higher cellular-modulated immunity than that of MBB birds (Cheng *et al.*, 2001b). In contrast, the MBB birds had higher levels of circulating immunoglobulin G and a higher ratio of circulating H:L (Cheng *et al.*, 2001b). These results further support the protective functions of CORT in permitting animals to remain alert and keep a stably physiological homeostasis in response to relevant stimulators.

Line differences in blood haematological parameters

Heterophils:lymphocytes ratio have be used as physiological indicators of stress in evaluation of chicken responsiveness to novel environments, especially, under chronic stress (Gross and Siegel, 1983; Maxwell, 1993; Siegel, 1995). The KGB birds had a lower numbers of heterophils and a lower H:L ratio. This result suggests that the KGB birds have a greater adaptive capability to stress than the birds of the MBB line (*Table 4*). This result is consistent with previous studies that documented the KGB birds exhibited better feather score, lower mortality, and higher reproduction in a socially crowded environment (Craig and Muir, 1996a, b). In addition, the KGB birds showed improved adaptation to various stressors such as social, handling, cold, and heat stress when compared to the control birds (Hester *et al.*, 1996a,c). The present findings suggest that the H:L ratio and heterophilia could be used as indicators for genetic selection of chicken strains with higher resistance to stress. However, caution is advised, although selecting for high group productivity resulted in altered H:L ratio, the reverse may not be true, *i.e.* selecting for altered H:L ratio may not change group productivity in the desired direction.

Retention of normal levels of circulating eosinophils is associated with resistance to stress (Woolaston *et al.*, 1996; Hohenhaus *et al.*, 1998) and changes in blood eosinophils appear as a genotypic or phenotypic hallmark of physiological and psychological stress reaction (Malyshev *et al.*, 1993; Hohenhaus *et al.*, 1998). Circulating eosinophils has been used as a genetic selection criterion for resistance of sheep to *trichostrongyle parasites* (Woolaston *et al.*, 1996) and used as a phenotypic marker of resistance to *nematode parasites* (Hohenhaus *et al.*, 1998). Those hypotheses are consistent with the present findings that eosinophilia was exhibited in the MBB birds but not in the KGB birds (*Table 4*). Selection induced different regulation of eosinophils could be associated with the selection related alterations of the neuroendocrine-immune system. A correlation has been established between alterations in blood eosinophils, corticosterone levels, and catecholamine metabolism (Malyshev *et al.*, 1985). As such, injection of adrenocorticotropin hormone induced eosinophilia in black steers (Ishizaki and Kariya, 1999) and hens (Gray *et al.*, 1989). Eosinopenia induced by handling and sampling can be blocked by both adrenalectomy and hypophysectomy in rats (Treloar, 1977).

Line differences in immunological parameters

The ratio of $CD4^{+}$:$CD8^{+}$ T cells has been used as an indictor to evaluate cell-mediated immune response. Previous studies reported that the normal ratio of $CD4^{+}$:$CD8^{+}$ T cells should be larger than 1.5, otherwise, cellular immune mechanisms are greatly impaired (Levinson and Jawetz, 1996) and survivability is reduced (Reid and Tervit, 1995). Compared to the MBB birds, the KGB birds may have a more efficient cellular-mediated immunity, since the $CD4^{+}$:$CD8^{+}$ ratio of circulating T cells was much higher in the KGB birds than that in the MBB birds (*Table 5*). In addition, the percentage of several cell types involved in the cellular-mediated immunity trended to be greater in the birds of the KGB line than in the MBB line, including monocytes (124%), and $\gamma\delta$ T cells (131%) (*Tables 4 and 5*). Similar to the present data, Bayyari *et al.*, (1997) reported that cell-mediated immunity was affected in turkeys selected for faster growth.

Plasma Immunoglobulin G was up-regulated in the MBB birds but not in the KGB birds (*Figure 2*). These results are consistent with the hypothesis that alteration in IgG synthesis is a common response in selection programmes to various stressors, such as social stress and infection (Siegel and Gross, 1980; Siegel *et al.*, 1982; Van der Zijpp *et al.*, 1983). The mechanisms underlying inherent higher IgG concentrations in the MBB birds could be related to the lower ratio of $CD4^{+}$:$CD8^{+}$ T cells. Although $CD4^{+}$ T cells help B cells to production of antibodies (T cell-dependent response), antibody production can also be suppressed by inhibitory lymphokines released by $CD4^{+}$ T cells (Levinson and Jawetz, 1996). In addition, the higher levels of IgG could be regulated by mediators without involvement of T cells (T cell-independent response), which does not required memory B cells (Levinson and Jawetz, 1996). Boa-Amposem and co-investigators (1999) have shown that in response to booster inoculation with sheep red blood cells (SBRC), the chickens of their HA line (high antibody response to SRBC line) exhibited significantly lower immunological memory than chickens of the LA line (low antibody response to SRBC line).

The different regulations of immunity between selected lines may be related to each line's unique endocrine system changes, such as DA and other catecholamines. Enhanced blood DA and catecholamines in the MBB birds may have suppressive effect on its immunity through binding to their receptors within immune organs and cells (Plaut, 1987). Previous studies have shown DA and EP as well as CORT suppress proliferations of T cells and B cells, reduced T cells and natural killer cells responsiveness to antigens, and inhibition of cytokine production (Brown-Borg *et al.*, 1993; Hessing *et al.*, 1995; Dunn, 1996; Wilkie and Mallard, 1999). In addition, differential regulation of immunity could be related to selection induced morpho-functional changes of lymph organs and immunological cells. These changes have been identified in previously selected chickens. For example, the LA chickens have larger thymuses, smaller spleens and bursas of Fabricius than the HA chickens (Ubosi *et al.*, 1985).

Conclusion

The present study demonstrates that there are line differences in the regulation of the adrenal system, CORT; monoamines, 5-HT, DA, EP and NE; and immunity, the subset of T-cells and leukocytes as well as production of Immunoglobulin G. These results suggest that group selection for higher productivity and longevity may have altered the neurochemical and endocrine systems resulting in better coping abilities to novel environments and greater resistance to stressors. The unique homeostatic characteristics of each selected line may provide a neurobiological basis for investigating effects of genetic factors on physiological functions of biogenic amines involved in productivity and longevity related to domestic behaviours. Understanding the mechanisms involved in the selection programme will facilitate the development of interventions aimed at improvement of animal well-being and productivity.

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Table 1 Genetic selection-induced alterations in the productivity and survivability in laying hens.

Trait	KGB line[1]	MBB line
Mortality, %	$1.3^b \pm 0.1$	$8.6^a \pm 0.5$
Longevity, d	$363^b \pm 0.4$	$193^a \pm 21$
Egg Number, per hen	$295^b \pm 11$	$108^a \pm 12$
Egg Mass, per hen, g/d	$48^b \pm 2$	$17^a \pm 1.8$
Egg Weight, g	$59.4^a \pm 0.6$	$58.9^a \pm 0.8$

[a, b]Means within a row with no common superscript differ significantly (P < 0.05).
[1]The KGB and MBB line were selected from high and low productivity and survivability resulting from cannibalism and flightiness. g, gram; and d, day.

Table 2 Selection-induced alterations in blood concentrations of catecholamines and serotonin in laying hens.

Lines[1]	Dopamine (ng/ml)	Epinephrine (ng/ml)	Norepinephrine (ng/ml)	EP:NE (%)	Serotonin (ng/ml)
KGB	0.59 ± 0.08^a	0.30 ± 0.06^a	0.86 ± 0.12	34.0^a	11.8 ± 0.07^a
MBB	2.42 ± 0.76^b	0.59 ± 0.13^b	0.84 ± 0.13	72.5^b	14.3 ± 0.06^b

[a,b]Means within a column with different superscript are statistically different (P < 0.01).
[1]The KGB and MBB line were selected from high and low productivity and survivability resulting from cannibalism and flightiness. EP, Epinephrine; and NE, Norepinephrine.

Table 3 Selection-induced alterations in the adrenal systems.

Lines	Adrenal glands (Index) [1]	Corticosterone (ng/ml)
KGB[2]	72 ± 4^a	1.87 ± 0.19
MBB	58 ± 5^b	1.49 ± 0.21

[a,b]Means within a column with different superscript are statistically different (n=12, P < 0.05).
[a]Adrenal weight index = the absolute adrenal gland weight/body weight x 100.
[2]The KGB and MBB lines were selected for high and low productivity and survivability resulting from cannibalism and flightiness.

Table 4 Selection-induced alterations in the differential leukocyte counts in laying hens

Line[1]	Heterophils (H)	Lymphocytes (L)	H:L ratio (x 100)	Monocytes	Eosinophils	Basophils
KGB	10.7 ± 1.1[b]	83.4 ± 1.3[a]	13.0[b]	2.6 ± 0.4	1.7 ± 0.2[b]	1.6 ± 1.1
MBB	20.4 ± 1.8[a]	72.3 ± 1.8[b]	29.4[a]	2.1 ± 0.4	3.8 ± 0.4[a]	1.4 ± 0.2

[a,b]Means within a column with no common superscript differ significantly ($P < 0.01$).
[1]The KGB and MBB lines were selected for high and low productivity and survivability resulting from cannibalism and flightiness.

Table 5 Selection-induced alterations in the subpopulations of T cells in the hens

Group[1]	$CD4^+$ cells (% positive)	$CD8^+$ cells (% positive)	$CD4^+$:$CD8^+$ (ratio)	γδ cells (% positive)
KGB	33.0 ± 2.1	18.2 ± 1.8[b]	1.9[a]	16.1 ± 2.1
MBB	29.3 ± 3.3	25.8 ± 1.5[a]	1.1[b]	12.2 ± 2.8

[a,b]Means within a column with no common superscript differ significantly ($P < 0.01$).
[1]The KGB and MBB lines were selected for high and low productivity and survivability resulting from cannibalism and flightiness.

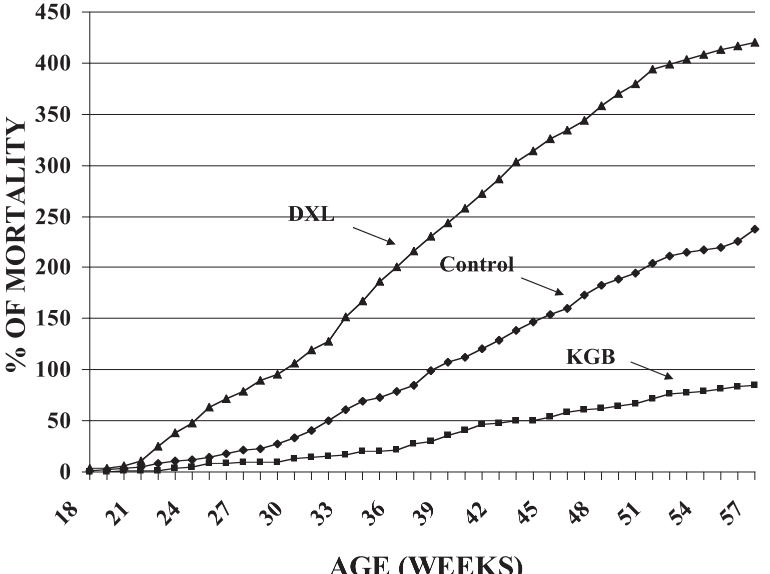

Figure 1a

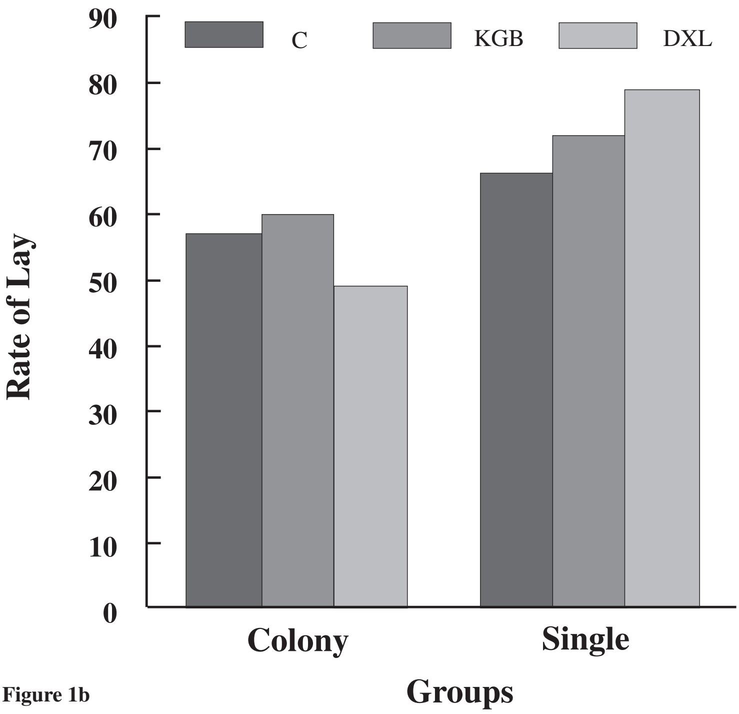

Figure 1b

Figure 1 a, Annual percent mortality of the commercial (Dekalb XL), control, and KGB birds housed in colony cages. Compared to birds from the Dekalb XL line and control line, selected KGB birds (kinder gentler bird) had the lowest mortality; and b, rate of egg production of the commercial (Dekalb XL), control, and KGB birds. Among the three lines, the selected KGB birds had the highest production in the colony cages, while Dekalb XL birds had the highest production in the single-bird cage. C, control; KGB, selected kinder gentler bird' and DXL, commercial Dekalb XL bird.

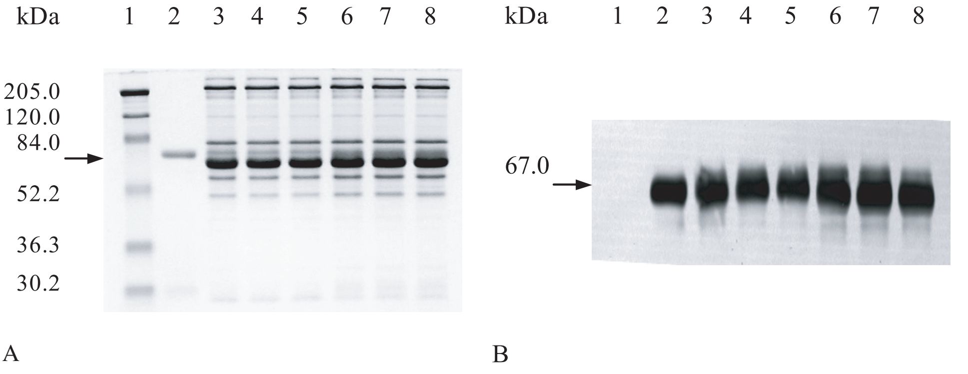

Figure 2 **A, separated proteins on the gels were stained with Coomassie blue, and B, separated protein on the gels were detected by the western blot analysis plus ECL. Lane 1, prestained broad range molecular weight marker; Lane 2, commercial chicken IgG; Lanes 3 to 5, samples from KGB hens, and Lanes 6 to 8, samples from MBB hens. Notes, the band from samples was identical with the band prepared with commercial chicken IgG, and size of IgG was identified approximately at 67 kDa (arrow). ECL = enhanced chemiluminescence.**